Plant Physiol. (1992) 99, 356–358
0032-0889/92/99/0356/03/$01.00/0

Received for publication October 29, 1991
Accepted February 10, 1992

Plant Gene Register

Comparison of the *rbcL* Gene Sequence of Two Potato Cultivars with Differential Sensitivity to Ozone[1]

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Rubisco (EC 1.1.1.39) is a key enzyme in photosynthetic carbon assimilation and catalyzes the first committed step of CO_2 fixation in the Calvin cycle. It has been estimated that nearly 70% of soluble leaf protein is in the form of Rubisco (4). Higher plant Rubisco is an oligomer composed of eight large subunits (M_r 52,000–55,000) and eight small subunits (M_r 12,000–15,000). The large subunit contains the active site for carboxylation and oxygenation reactions, and the small subunit protein is hypothesized to maintain the apoenzyme in a stable conformation capable of activation to a catalytically active holoenzyme (5). The small subunit protein of Rubisco is nuclear encoded by the *rbcS* gene which exists as a multigene family (2). In contrast, the Rubisco large subunit protein is encoded by the *rbcL* gene situated on the chloroplastic DNA (5). The *rbcL* gene exists as a single copy and contains no introns. Currently, no information exists concerning the *rbcL* nucleotide sequence of potato (*Solanum tuberosum*). The objective of this study was to investigate *rbcL* to identify differences in the nucleotide sequence for Rubisco from cultivars of potato previously determined to be sensitive (cv Cherokee) and tolerant (cv Superior) to ozone (1, 6). The mechanism for differential genotypic responses to ozone is unknown. One explanation may be the vulnerability of SH side groups of Rubisco to oxidation (9). The oxidation of SH groups is known to render Rubisco catalytically inactive and more susceptible to proteolysis (7).

Chloroplast DNA was isolated from both potato cultivars and screened with the *rbcL* gene from maize (Table I). The nucleotide and predicted amino acid sequences for *rbcL* from potato cultivars Cherokee and Superior were found to be identical (Fig. 1). Consequently, the differential sensitivity to ozone between cultivars could not be explained by the absolute number of Cys residues because each nucleotide sequence predicts the occurrence of nine SH groups per large subunit protein (Fig. 1). For comparison purposes, the deduced amino acid sequence of tobacco (8) and spinach (10) *rbcL* genes are shown (Fig. 1). It is still possible that the difference in the Rubisco response can be explained by characteristics of the *rbcS* gene(s) and/or expression of these genes.

Table I. *Characteristics of Gene rbcL from Solanum tuberosum Chloroplast*

Organism:
Solanum tuberosum L. (cv Superior and cv Cherokee).
Gene; Function:
rbcL; encodes large subunit of Rubisco (EC 4.1.1.39).
Techniques:
Chloroplast DNA isolation, probed chloroplast DNA using heterologous 2.6-kilobase *rbcL* fragment from *Zea mays* L. obtained from plasmid pZmB1A (3). Isolated 3.9-kilobase *Eco*RI fragment containing entire *Solanum rbcL* gene, subcloned restriction fragments into pBluescript phagemids KS+ and KS− (Stratagene, La Jolla, CA) and dideoxy sequencing of both strands.
Method of Identification:
Sequence comparison to published tobacco large subunit sequence (8). *rbcL* from tobacco contains 1434 base pairs and possess 98% identity to potato *rbcL*.
Features of Gene Structure:
rbcL for both Superior and Cherokee cultivars was 1434 base pairs in length and identical in nucleotide sequence. Nine Cys residues were present in the coding region (underlined, Fig. 1). The Cys residues were encoded by five TGT codons and 4 TGC codons.
Codon Usage:
Codons not used are CTC (L), TCG (S), AGG (R); G or C in the third codon position occurs at frequency of 30.8%.
(G + C) Content:
43.9% in protein-coding region.
Structural Features of Protein:
Open reading frame consists of 477 amino acids; predicted M_r 52,857; no amino acid accounts for >10% of the total amino acids.
Antibodies:
Antisera to holoenzyme Rubisco available.
Subcellular Location:
Chloroplast; assembled with small subunit protein.
EMBL Accession No.:
M76402

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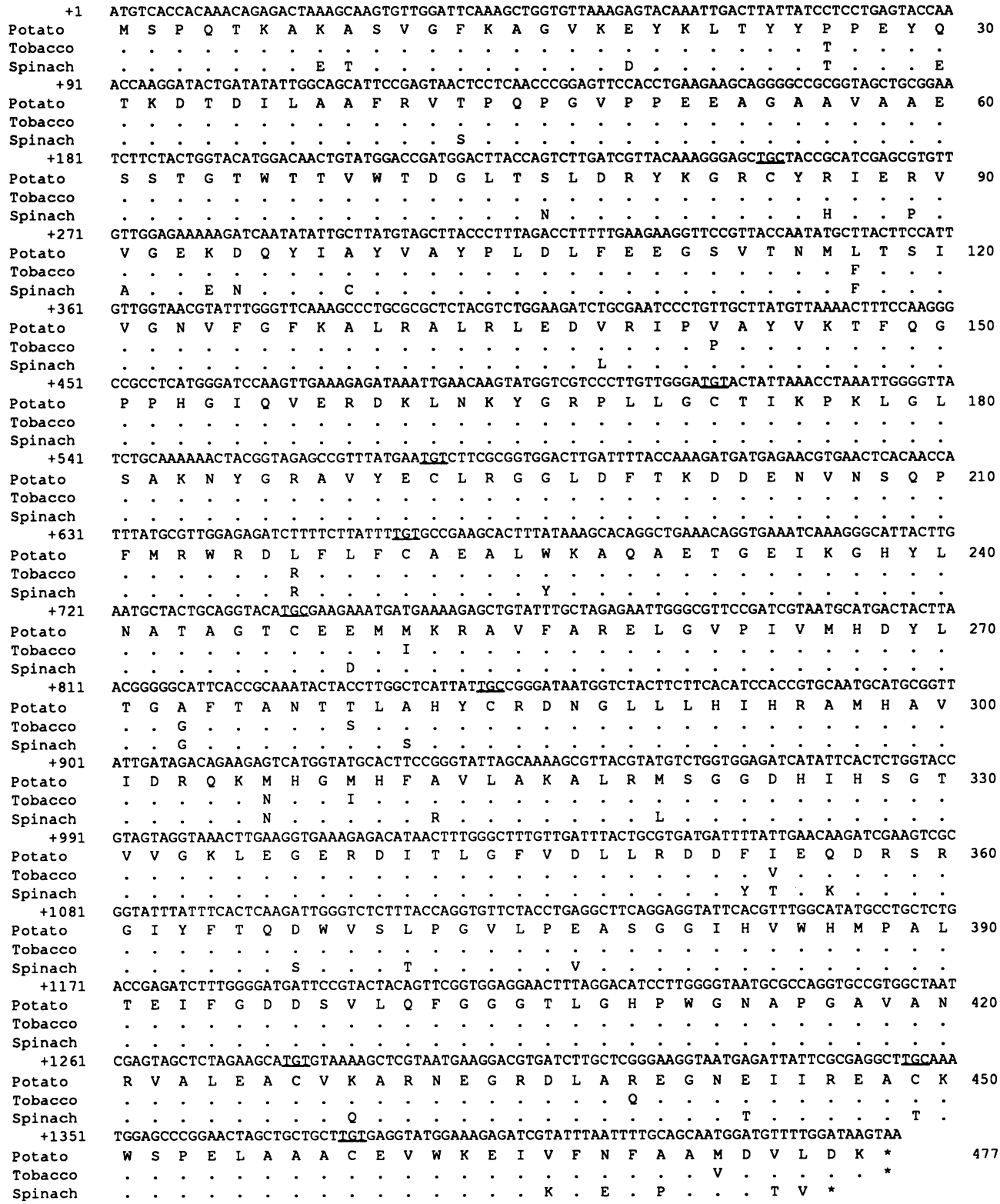

Figure 1. Nucleotide sequence and deduced amino acid sequence of *rbcL* from potato. This sequence was identical for cultivars Cherokee and Superior. The open reading frame for potato *rbcL* extends from positions +1 to +1431 and encodes a polypeptide of 477 amino acids. The TAA stop codon is marked by an asterisk. The location of nine predicted Cys amino acid residues (underlined) is shown. For comparison, the alignment of the amino acid sequences of the Rubisco large subunit from tobacco (8) and spinach (10) are shown.

ACKNOWLEDGMENTS

We gratefully acknowledge Dr. Ming Tien for providing us with technical advice and Dr. Anthony Gatenby for providing us with the pZmB1A plasmid.

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